(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 144 623 B9

(12) # KORRIGIERTE EUROPÄISCHE PATENTSCHRIFT

Hinweis: Bibliographie entspricht dem neuesten Stand

(15) Korrekturinformation:
Korrigierte Fassung Nr. 1 (W1 B1)
Korrekturen, siehe Seite(n) 2, 3, 11, 14, 15

(48) Corrigendum ausgegeben am:
05.03.2003 Patentblatt 2003/10

(45) Veröffentlichungstag und Bekanntmachung des Hinweises auf die Patenterteilung:
28.08.2002 Patentblatt 2002/35

(21) Anmeldenummer: **00910510.7**

(22) Anmeldetag: **29.01.2000**

(51) Int Cl.[7]: **C12N 15/11**, A61K 31/713

(86) Internationale Anmeldenummer:
PCT/DE00/00244

(87) Internationale Veröffentlichungsnummer:
WO 00/044895 (03.08.2000 Gazette 2000/31)

(54) **VERFAHREN UND MEDIKAMENT ZUR HEMMUNG DER EXPRESSION EINES VORGEGEBENEN GENS**

METHOD AND MEDICAMENT FOR INHIBITING THE EXPRESSION OF A DEFINED GENE

METHODE ET MEDICAMENT DESTINES A INHIBER L'EXPRESSION D'UN GENE DONNE

(84) Benannte Vertragsstaaten:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priorität: **30.01.1999 DE 19903713**
24.11.1999 DE 19956568

(43) Veröffentlichungstag der Anmeldung:
17.10.2001 Patentblatt 2001/42

(60) Teilanmeldung:
02003683.6 / 1 214 945

(73) Patentinhaber: **Ribopharma AG**
95440 Bayreuth (DE)

(72) Erfinder:
- **KREUTZER, Roland**
  95466 Weidenberg (DE)
- **LIMMER, Stephan**
  95447 Bayreuth (DE)

(74) Vertreter: **Gassner, Wolfgang, Dr.**
Patentanwalt
Nägelsbachstrasse 49a
91052 Erlangen (DE)

(56) Entgegenhaltungen:
WO-A-92/19732
WO-A-98/05770
WO-A-99/32619

- **UHLMANN E ET AL: "ANTISENSE OLIGONUCLEOTIDES: A NEW THERAPEUTIC PRINCIPLE" CHEMICAL REVIEWS,US,AMERICAN CHEMICAL SOCIETY. EASTON, Bd. 90, Nr. 4, 1. Juni 1990 (1990-06-01), Seiten 543-584, XP000141412 ISSN: 0009-2665**
- **MADHUR K. ET AL.: "Antisense RNA : function and fate of duplex RNA in cells of higher eukaryotes." MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Bd. 62, Dezember 1998 (1998-12), Seiten 1415-1434, XP000909741**

EP 1 144 623 B9

EP1144623 GRANTED CLAIMS - ENGLISH

International Patent Application No. PCT/DE00/00244
of Dr Roland Kreutzer and Dr Stefan Limmer

New Patent Claims

1. Method for inhibiting the expression of a given target gene in a cell in vitro, where an oligoribonucleotide with double-stranded structure (dsRNA) formed by two separate RNA single strands is introduced into the cell, where one strand of the dsRNA has a region which is complementary to the target gene,
characterized in that
the complementary region has less than 25 successive nucleotide pairs.

2. Method according to claim 1, where the dsRNA is enclosed by micellar structures, preferably by liposomes.

3. Method according to either of the preceding claims, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

4. Method according to one of the preceding claims, where the target gene is expressed in eukaryotic cells.

5. Method according to one of the preceding claims, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

6. Method according to one of the preceding claims, where the target gene is expressed in pathogenic organisms, preferably in plasmodia.

International Patent Application No. PCT/DE00/00244
of Dr Roland Kreutzer and Dr Stefan Limmer

New Patent Claims

1. Method for inhibiting the expression of a given target gene in a cell in vitro, where an oligoribonucleotide with double-stranded structure (dsRNA) formed by two separate RNA single strands is introduced into the cell, where one strand of the dsRNA has a region which is complementary to the target gene,
characterized in that
the complementary region has less than 25 successive nucleotide pairs.

2. Method according to claim 1, where the dsRNA is enclosed by micellar structures, preferably by liposomes.

3. Method according to either of the preceding claims, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

4. Method according to one of the preceding claims, where the target gene is expressed in eukaryotic cells.

5. Method according to one of the preceding claims, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

6. Method according to one of the preceding claims, where the target gene is expressed in pathogenic organisms, preferably in plasmodia.

7. Method according to one of the preceding claims, where the target gene is part of a virus or viroid.

8. Method according to claim 7, where the virus is a virus or viroid which is pathogenic for humans.

9. Method according to claim 7, where the virus or viroid is a virus or viroid which is pathogenic for animals or phytopathogenic.

10. Method according to one of the preceding claims, where segments of the dsRNA are in double-stranded form.

11. Method according to one of the preceding claims, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

12. Method according to one of the preceding claims, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

13. Method according to one of the preceding claims, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

14. Method according to one of the preceding claims, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

15. Method according to one of the preceding claims, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

16. Method according to one of the preceding claims, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

17. Method according to one of the preceding claims, where the chemical linkage is formed by azabenzene units introduced into the double-stranded structure.

18. Method according to one of the preceding claims, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

19. Method according to one of the preceding claims, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxyl-benzoyl)cystamine; 4-thiouracil; psoralene.

20. Method according to one of the preceding claims, where the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

21. Method according to one of the preceding claims, where the chemical linkage at the ends of the double-stranded structure is formed by triple-helix bonds.

22. Method according to one of the preceding claims, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

23. Method according to one of the preceding claims, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

24. Method according to one of the preceding claims, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

25. Method according to one of the preceding claims, where the coat protein is derived from polyomavirus.

26. Method according to one of the preceding claims, where the coat protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

27. Method according to one of the preceding claims, where, when a capsid or capsid-type structure is formed from the coat protein, one side faces the interior of the capsid or capsid-type structure.

28. Method according to one of the preceding claims, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

EPA/EPO/OEB
D-80298 München
+49 89 2399-0
TX 523 656 epmu d
FAX +49 89 2399-4465

Europäisches Patentamt | European Patent Office | Office européen des brevets

Generaldirektion 2 | Directorate General 2 | Direction Générale 2

Gassner, Wolfgang, Dr.
Patentanwalt
Nägelsbachstrasse 49a
91052 Erlangen
ALLEMAGNE

Datum/Date
18/07/02

Zeichen/Ref./Réf.	Anmeldung Nr./Application No./Demande n°./Patent Nr./Patent No./Brevet n°.
411758GA	00910510.7-2107 1144623

Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire
Ribopharma AG

ENTSCHEIDUNG ÜBER DIE ERTEILUNG EINES EUROPÄISCHEN PATENTS GEMÄSS ART. 97(2) EPÜ

Nach Prüfung der europäischen Patentanmeldung Nr. 00910510.7 wird für die benannten Vertragsstaaten ein europäisches Patent mit der Bezeichnung und mit den Unterlagen erteilt, die in der gemäss Regel 51(4) EPÜ ergangenen Mitteilung vom 26.11.01 aufgeführt sind. Den hierzu gegebenenfalls beantragten bzw. vereinbarten Änderungen hat die Prüfungsabteilung zugestimmt. Die vom Anmelder n a c h Eingang der Mitteilung gem. Regel 51(6) EPÜ beantragten und am 21.05.02 beim EPA eingegangenen Berichtigungen wurden berücksichtigt.

Patentnummer : 1144623
Anmeldetag : 29.01.00
Beanspruchte Priorität : 30.01.99/DE 19903713
24.11.99/DE 19956568

Benannte Vertragsstaaten
und Patentinhaber : AT-BE-CH-CY-DE-DK-ES-FI-FR-GB-GR-IE-IT-LI-LU-MC-NL-PT-SE
Ribopharma AG
Universitätsstrasse 30
95440 Bayreuth/DE

Die Entscheidung wird an dem Tage wirksam, an dem im Europäischen Patentblatt auf die Erteilung hingewiesen worden ist (Art.97(4) und (5) EPÜ).

Der Hinweis über die Erteilung wird im Europäischen Patentblatt 02/35 am 28.08.02 bekanntgemacht.

Prüfungsabteilung
LUDWIG G H E HARS J MERCKLING V

Einschreiben

(19) Canadian Intellectual Property Office
An Agency of Industry Canada

Office de la Propri,t, Intellectuelle du Canada
Un organisme d'Industrie Canada

(11) CA 2 359 180 (13) A1

(40) 03.08.2000
(43) 03.08.2000

(12)

(21) 2 359 180

(22) 29.01.2000

(51) Int. Cl.7: C12N 15/11, A61K 31/713

(85) 18.07.2001

(86) PCT/DE00/00244

(87) WO00/44895

(30) 199 03 713.2 DE 30.01.1999
199 56 568.6 DE 24.11.1999

(71)
KREUTZER, ROLAND,
Glotzdorf 26, D-95466, WELDENBERG, XX (DE).
LIMMER, STEPHAN,
Leibnizstrasse 14
D-95447, BAYREUTH, XX (DE).

(72)
KREUTZER, ROLAND (DE).
LIMMER, STEPHAN (DE).

(74)
FETHERSTONHAUGH & CO.

(54) METHODE ET MEDICAMENT DESTINES A INHIBER L'EXPRESSION D'UN GENE DONNE
(54) METHOD AND MEDICAMENT FOR INHIBITING THE EXPRESSION OF A GIVEN GENE

(57)
[222]The invention relates to a medicament containing at least one double-stranded [2]oligoribonucleotide (dsRNA) designed to inhibit the expression of a target [2]gene. According to the invention, one strand of the dsRNA is at least in part [2]complementary to the target gene.[2]

CANADIAN EQUIVALENT OF EP 1144623